JOURNAL OF VIROLOGY, Nov. 1994, p. 7586–7590
0022-538X/94/$04.00+0

Vol. 68, No. 11

Analysis of the Contributions of Herpes Simplex Virus Type 1 Membrane Proteins to the Induction of Cell-Cell Fusion

NICHOLAS DAVIS-POYNTER,† SUSANNE BELL, TONY MINSON, AND HELENA BROWNE*

Division of Virology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom

Received 13 June 1994/Accepted 11 August 1994

The contributions of a set of herpes simplex virus type 1 membrane proteins towards the process of cell-cell fusion were examined with a series of deletion mutants into which a syncytial mutation had been introduced at codon 855 of the glycoprotein B (gB) gene. Analysis of the fusion phenotypes of these recombinant viruses in Vero cells revealed that while gC, gG, US5, and UL43 are dispensable for syncytium formation at both high and low multiplicities of infection, gD, gHgL, gE, gI, and gM were all required for the fusion of cellular membranes. These data confirm that the requirements for virion entry and cell-cell fusion are not identical. gD and gHgL, like gB, are essential for both processes. gE, gI, and gM, on the other hand, are dispensable for virus penetration, yet play a role in cell-to-cell spread by the direct contact route, at least on an SC16 gB^{ANG} background.

The entry of herpes simplex virus (HSV) into the host cell requires the fusion of the virus envelope with the cell membrane, a process that is thought to occur at the cell surface or in an early endosome. Current data point to an essential role for glycoprotein B (gB), glycoprotein D (gD), and the glycoprotein H-glycoprotein L (gHgL) complex in the fusion process. The best evidence for this role comes from studies of the phenotype of deletion mutants lacking individual glycoproteins: virions devoid of gB, gD, or gHgL bind to cells but fail to penetrate, and this block may be overcome, albeit inefficiently, with the artificial fusogen polyethylene glycol (4, 10, 15). Supporting evidence is provided by studies which show that neutralizing antibodies directed against these proteins block viral penetration but do not prevent virus attachment. It is uncertain whether gB, gD, or gHgL is sufficient alone to mediate penetration. Analysis of the phenotype of viruses which lack other viral membrane proteins has established that gC is required for efficient binding but not for penetration (12) and that mutants lacking gE, gI, gG, gM, or the US5 or UL43 gene products are viable and have normal particle/infectivity ratios by comparison with their parental virus strains (2, 17). However, the properties of mutants lacking other potential membrane proteins have yet to be examined in detail, and there is evidence that a tegument protein may also be involved in penetration (1).

Transfer of HSV type 1 (HSV-1) from the infected cell to uninfected neighbors, for example, in plaque formation, can occur in the presence of neutralizing antibody and is thought to involve limited fusion of the plasma membranes of infected and uninfected cells. The extensive cell fusion caused by syncytial strains is assumed to be an uncontrolled form of this process, and the UL53 (gK), UL27 (gB), UL20, and UL24 gene products are thought to be modulators of cell-cell fusion (24). It is likely that aspects of the fusion of the virion envelope with its target cell membrane will have features in common with cell-cell fusion; thus, gB and gD are required for HSV-1 entry and are also required for HSV-1-induced cell-cell fusion since deletion of the gB or gD genes in a syncytial virus abolishes polykaryocyte formation (4, 15). It is equally apparent, however, that the two processes are not identical, a view supported most dramatically by studies of the related α-herpesvirus, pseudorabies virus, which show that the gD homolog (gp50) is essential for virus entry but is not required for cell-to-cell spread (20). Conversely, the gEgI complex of HSV-1 and the homologous complex of pseudorabies virus are dispensable for virion entry but are required for efficient cell-to-cell spread and for cell-cell fusion (2, 9, 28). Comparison of the viral proteins required for induction of virus-cell fusion and cell-cell fusion should give an indication of the differences and similarities between the two processes. Many studies have implicated individual HSV-1 glycoproteins in cell-cell fusion. Antibodies to gH, gD, and gE have been shown to inhibit cell fusion by syncytial strains (6, 11, 18), and deletion mutants constructed in syncytial strains but lacking gB, gD, gE, or gI are reported to lose the cell fusion phenotype (2, 5, 15). In contrast, mutants lacking gC remain syncytial (22). It is difficult, nevertheless, to build a coherent view of the proteins involved in cell fusion because different mutants have been constructed in different parental virus strains and because some reports are conflicting or ambiguous. Thus, Neidhardt et al. (19) reported the construction of a gE-negative mutant that retained a syncytial phenotype, a result which contrasts with that reported by Balan et al. (2), while a gD-negative mutant that had lost the ability to induce fusion (15) lacked an intact gene for gI, a protein whose function appears to be required for expression of the syncytial phenotype (2). The main objective of this study was to assess the contribution of different HSV-1 membrane proteins to the induction of cell-cell fusion by examining the phenotype of a series of deletion mutants constructed in a single syncytial virus background.

The construction of a syncytial strain of HSV-1 SC16 which contains a substitution of valine for alanine 855 in the gB gene has been described previously (2), and since this mutation is found in the ANG strain of HSV-1 (25), the resulting virus was named SC16 gB^{ANG}. Individual genes encoding membrane proteins were disrupted in this virus by insertion of a *lacZ*

* Corresponding author. Mailing address: Division of Virology, Department of Pathology, University of Cambridge, Tennis Court Rd., Cambridge CB2 1QP, United Kingdom. Phone: (223) 336921. Fax: (223) 336926.

† Present address: Department of Microbiology, University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands, WA 6009, Australia.

TABLE 1. Virus nomenclature

Virus	Abbreviated nomenclature
SC16gB^{ANG} ΔUL22.lacZ	gH$^-$ B^{ANG}
SC16gB^{ANG} ΔUS6.lacZ	gD$^-$ B^{ANG}
SC16gB^{ANG} ΔUL44.gus	gC$^-$ B^{ANG}
SC16gB^{ANG} ΔUS8.lacZ	gE$^-$ B^{ANG}
SC16gB^{ANG} ΔUS7.lacZ	gI$^-$ B^{ANG}
SC16gB^{ANG} ΔUS4.lacZ	gG$^-$ B^{ANG}
SC16gB^{ANG} ΔUL10.lacZ	gM$^-$ B^{ANG}
SC16gB^{ANG} ΔUS5.lacZ	US5$^-$ B^{ANG}
SC16gB^{ANG} ΔUL43.lacZ	UL43$^-$ B^{ANG}

or β-glucuronidase expression cassette. Thus, SC16gB^{ANG} ΔUL10.lacZ is an SC16gB^{ANG} derivative which lacks gM by virtue of disruption of the UL10 open reading frame by insertion of a *lacZ* expression cassette. A simplified version of this nomenclature is presented in Table 1, and all viruses will be referred to by their abbreviated names in the rest of this report. HSV-1 mutants in which gH, gG, gE, gI, or gJ (US5) coding sequences are disrupted have been described previously (2, 10). A gD-negative derivative of SC16 was constructed by insertion of the human cytomegalovirus immediate-early promoter and β-galactosidase coding sequences (derived as described in reference 10) between the *Pvu*II restriction sites of the HSV-1 genome at nucleotides 138072 and 138574. This virus was propagated on the complementing Vero-derived cell line VD60, described in reference 15. A gC-negative derivative of SC16 was derived by insertion of β-glucuronidase coding sequences as a 1.88-kb *Bam*HI-*Eco*RI fragment of plasmid pGUS-1 (a gift from G. Murphy, Plant Breeding Institute, Norwich, United Kingdom) under control of the human cytomegalovirus immediate-early promoter, into the gC gene between nucleotides 96751 and 97649 of the HSV genome. Each of these mutants was crossed with SC16gB^{ANG} by coinfection at a multiplicity of infection (MOI) of 5 in Vero cells or BHK cells or, in the case of the gH$^-$ and gD$^-$ mutants, in the relevant helper cell line (F6 and VD60, respectively). The progeny were plated on the same cell lines, and monolayers were stained for β-galactosidase (as described in reference 10) or β-glucuronidase by using 1 mM β-D-glucuronide glucuronosohydrolase in a 1% agarose overlay. Positive plaques were picked and subjected to two further rounds of plaque purification. For construction of viruses with deletions in gM and UL43, SC16 gB^{ANG} DNA was cotransfected with deletion plasmids pC78.1 (UL10-*lacZ*) linearized with *Sca*I and pC75.1 (UL43-*lacZ*) linearized with *Xba*I (16), by the method of Chen and Okayama (7). Since the simian virus 40 early promoter present in these constructs was found to function poorly in Vero cells, transfection progeny were replated on BHK21 cells, and after being stained with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), blue plaques were picked and subjected to two further rounds of plaque purification. The genomes of all recombinant viruses were analyzed by Southern hybridization to confirm both the presence of the Ang mutation in the gB locus and the deletion of the particular glycoprotein gene under investigation.

Viruses deleted in the genes for the essential glycoprotein gH or gD, and with the Ang mutation present in the cytoplasmic tail of gB, were plated on Vero cell monolayers or on the respective complementing cell lines at 0.1 PFU per cell. After 2 days, the cells were fixed and stained with X-Gal. While both gH$^-$ B^{ANG} and gD$^-$ B^{ANG} produced large syncytia on F6 and

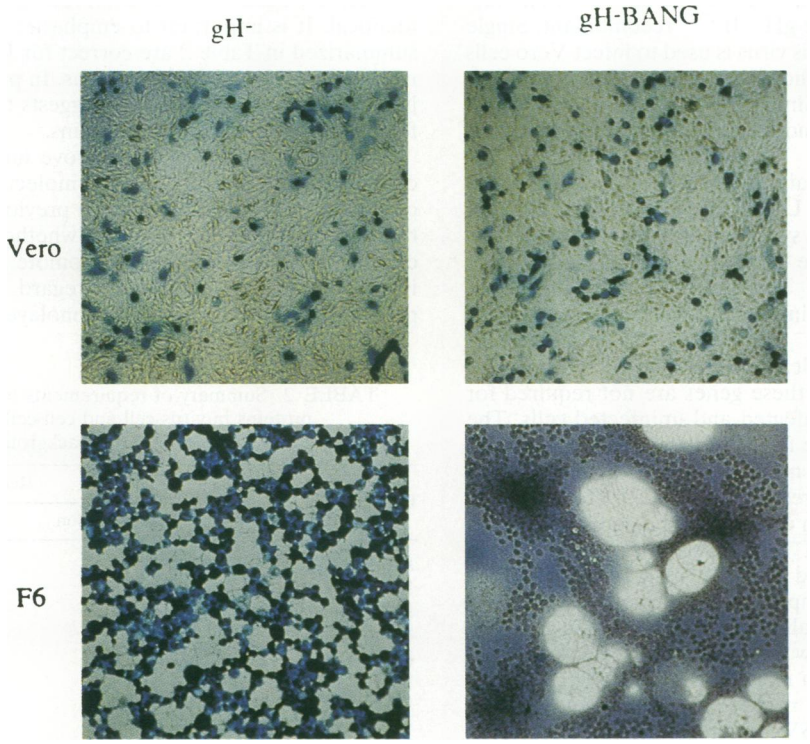

FIG. 1. The effect of deleting gH on syncytium formation. Monolayers of Vero or F6 cells were infected at an MOI of 0.1 with either a nonsyncytial gH-negative virus (gH$^-$) or recombinant virus gH$^-$ B^{ANG}. After 2 days, the monolayers were fixed and stained for β-galactosidase.

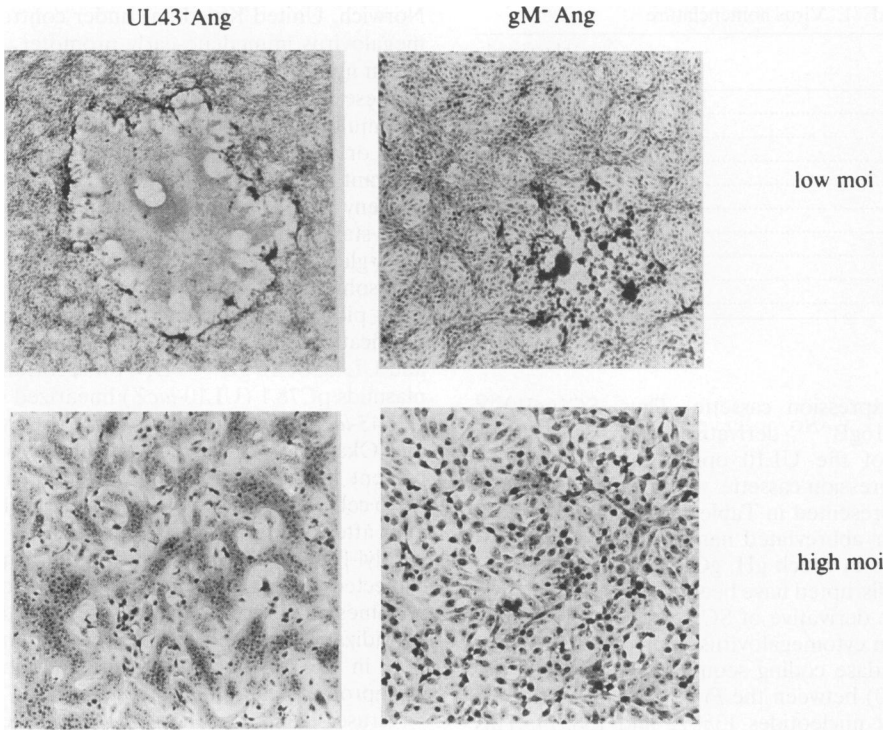

FIG. 2. Phenotypes of UL43- and gM-deficient syncytial viruses. Vero cells were infected with UL43$^-$ B^{ANG} or gM$^-$ B^{ANG} at either a low MOI (200 PFU per 5-cm dish) or a high MOI (10 PFU per cell). The low-MOI experiments were fixed and stained with 0.1% neutral red 2 days after infection. High-MOI experiments were fixed and stained 9 h after infection.

VD60 cell lines, respectively, both viruses failed to spread beyond the initial infected cell when plated on Vero monolayers as shown in Fig. 1 for the gH$^-$ B^{ANG} recombinant. Single blue cells are evident when this virus is used to infect Vero cells at both high and low MOIs, showing that the gHgL complex is essential for cell-cell fusion. Similar results were observed with gD$^-$ B^{ANG}, which failed to induce cell fusion on Vero, BHK, or MRC5 monolayers.

Recombinant viruses containing the gB^{ANG} mutation, together with deletions in the US4 (gG), UL44 (gC), US5, or UL43 gene, produced large syncytial plaques on Vero cells which were indistinguishable from those produced by the parent virus, SC16 gB^{ANG}. The plaque morphology of a UL43$^-$ B^{ANG} virus is shown in Fig. 2; those produced by gC$^-$ B^{ANG}, US5$^-$ B^{ANG}, and gG$^-$ B^{ANG} looked identical. These data imply, therefore, that at least on an SC16 gB^{ANG} syncytial background, the products of these genes are not required for membrane fusion between infected and uninfected cells. The finding that gC is dispensable for cell fusion is consistent with the observation that syncytial mutants often contain secondary mutations which abolish expression of gC (27). However, the effect on syncytium formation of deleting the genes for gE, gI, or gM (shown in Fig. 2 for gM$^-$ B^{ANG} and in the work of Balan et al. [2] for gE$^-$ B^{ANG} and gI$^-$ B^{ANG}) indicates that the products of these genes are important for the spread of virus by the cell-cell contact route: all three viruses produce small, poorly fused plaques and show a dramatically decreased extent of syncytium formation when examined 9 h after high-multiplicity infection of Vero cell monolayers. A summary of the contributions of this set of HSV-1 membrane proteins towards cell-cell fusion is presented in Table 2, together with their requirements for virion entry, and this highlights the finding that the two processes of membrane fusion, while having some similarities, such as a need for gB, gD, and gHgL, are not identical. It is important to emphasize that, while the results summarized in Table 2 are correct for HSV-1 SC16, they may not hold true for all HSV-1 strains. In particular, the report by Neidhardt et al. (19) strongly suggests that gE is not essential for cell fusion in some virus strains.

The experiments described above indicated that the gHgL complex and gD are essential molecules for the fusion of cellular membranes, as had been previously shown for gB (5), but do not address the issue of whether these molecules are capable of acting in *trans* to promote membrane fusion. To investigate this possibility with regard to the essential glycoproteins gHgL, gB, and gD, monolayers of Vero cells were

TABLE 2. Summary of requirements for HSV-1 membrane proteins in virus-cell and cell-cell fusion on an SC16 gB^{ANG} background

Protein	Required for:	
	Cell-cell fusion	Virion entry
gB	+	+
gHgL	+	+
gD	+	+
gE	+	−
gI	+	−
gM	+	−
gC	−	−
US5	−	−
UL43	−	−
gG	−	−

TABLE 3. Mixed infected cell populations used to determine whether gHgL, gD, and gB can operate in *trans* to promote cell fusion

Cell A		Cell B		Question
Infected with	Surface glycoproteins	Surface glycoproteins	Infected with	
gH^- B^{ANG}	gD gB^{ANG}	gB^{ANG} gHgL	gD^- B^{ANG}	Can gD and gHgL cooperate in *trans* in the presence of gB?
gB^- (KO82)	gD gHgL	gHgL gB^{ANG}	gD^- B^{ANG}	Can gD and gB cooperate in *trans* in the presence of gHgL?
gB^- (KO82)	gD gHgL	gD gB^{ANG}	gH^- B^{ANG}	Can gHgL and gB cooperate in *trans* in the presence of gD?

infected with either gH^- B^{ANG}, gD^- B^{ANG}, or a gB-null virus, KO82 (5), at a multiplicity of 10 PFU per cell. After 1 h, the cells were removed by trypsinization, pelleted, and resuspended in minimal essential medium containing 10% fetal calf serum. The two populations of cells were mixed at a range of ratios, 1:1, 1:10, and 1:100, and replated on 5-cm dishes. A summary of the mixed infected cell populations examined and of the essential glycoproteins present on their respective cell surfaces is presented in Table 3. The monolayers of mixed cells were incubated for a further 9 or 24 h, after which time they were examined for evidence of polykaryocyte formation. In a number of independent experiments of this kind, we saw no evidence of syncytium formation, at either 9 or 24 h after replating the mixed infected cell populations, suggesting that for fusion of cellular membranes by HSV, gHgL, gB, and gD must be present on the same cellular membrane and appear to act in *cis* during the fusion process. This is in contrast to the fusion mechanism of parainfluenza virus, in which expression of the F and HN proteins on different cells can lead to interactions which induce cell fusion (13).

The analysis of mutant viruses in which glycoprotein genes are deleted on a syncytial genetic background is a means of identifying HSV membrane proteins which are involved in cell-to-cell fusion but does not allow the determination of the minimum combination of molecules required for this process. We chose to address this question by coexpression of combinations of HSV-1 glycoproteins with vaccinia virus vectors, since this approach has proved successful for studying the fusion proteins F and HN of paramyxoviruses (13, 21). A panel of recombinant vaccinia viruses expressing gH, gL, gD, gB, gK, gE, and gI was constructed, as well as recombinants expressing syncytial forms of gB (the gB^{ANG} mutation) and gK (mutation of alanine 40 to either valine or threonine). In all the vaccinia virus recombinants, expression of the HSV glycoprotein gene was driven by the 4b late vaccinia virus promoter, and expression of the foreign gene was confirmed in all cases by either immunoprecipitation or immunoblotting with appropriate antibodies. Vero cells were infected with combinations of vaccinia viruses at 5 PFU per cell for each recombinant, and after the cells were washed three times with minimal essential medium they were either incubated for 9 h or harvested 1 h after infection, serially diluted, replated on uninfected monolayers of Vero cells, and incubated for 9 h. After the incubation period, the cells were examined for evidence of syncytium formation. We failed to observe polykaryocytes in any of these experiments but remain cautious about interpreting these findings; vaccinia virus may be an inappropriate vector for inducing cell fusion with these glycoproteins and may itself inhibit syncytium formation in this system, the levels of synthesis of the components of the fusion process may be critical and impossible to recreate with vaccinia virus, and it is also possible that HSV membrane proteins other than those which we have expressed are required to promote the fusion of cellular membranes. In particular, we have not yet included gM in these coexpression experiments because a satisfactory recombinant has yet to be made. Finally, it is worth noting that others have failed to induce syncytium formation by coexpression of HSV glycoproteins in other vector systems, including adenovirus (24).

The results of this study reinforce the view that the two processes of fusion of the HSV envelope with a cellular membrane and fusion of an infected cell membrane with its neighbor, while sharing some features, are not strictly analogous: gHgL, gD, and gB are essential both for virion entry and for cell-to-cell spread by the direct contact route, while gE, gI, and gM are involved only in the latter process (mutants deficient in gE, gI, or gM produce small plaques but are able to penetrate cells at rates equivalent to those of the wild type [2, 8, 17]). gG, gC, US5, and UL43 are not required for either process. Whether these requirements are identical on other syncytial genetic backgrounds and on other cell types remains to be determined, as does the molecular basis for these two forms of fusion. Although it is possible that those membrane proteins which are required for fusion are exerting their effects directly, they may equally be involved in indirect interactions which influence other essential molecules. It is not surprising that virion-cell fusion and cell-cell fusion have different requirements, given the differences between the types of membranes involved. Virion envelopes differ from plasma membranes in their degree of curvature and lipid and cholesterol composition, and the concentrations of HSV proteins present in the two membranes are likely to be quite different; indeed, even though gH is essential for both virus entry and cell fusion, it is possible to generate mutations in gH which can affect syncytium formation and yet have no effect on virus infectivity (26). We have no understanding of the nature of cellular molecules which interact with virus proteins to promote membrane fusion, although a role for heparin sulfate has recently been proposed (23). It has also been suggested that the gEgI complex, which is important for the spread of virus by the direct cell contact route and which is known to possess Fc-binding activity (3, 14), may function in fusion by interacting with cell surface proteins which are members of the immunoglobulin G supergene family (9). However, such molecules, which no doubt contribute to the cell type specificity of HSV-induced fusion, remain to be identified.

We thank Christine MacLean, Institute of Virology, Glasgow, United Kingdom, for plasmids pC78.1 and pC75.1 and G. Murphy for plasmid pGUS-1.

This work was supported by the Wellcome Trust UK.

REFERENCES

1. **Addison, C., F. J. Rixon, J. W. Palfreyman, M. O'Hara, and V. G. Preston.** 1984. Characterization of a herpes simplex virus type 1 mutant which has a temperature-sensitive defect in penetration of cells and assembly of capsids. Virology **138:**246–259.
2. **Balan, P., N. Davis-Poynter, S. Bell, H. Atkinson, H. Browne, and T. Minson.** 1994. An analysis of the *in vitro* and *in vivo* phenotypes of mutants of herpes simplex virus type 1 lacking glycoproteins gG, gE, gI or the putative gJ. J. Gen. Virol. **75:**1245–1258.
3. **Bell, S., M. Cranage, L. Borysiewicz, and T. Minson.** 1990. Induction of immunoglobulin G Fc receptors by recombinant vaccinia viruses expressing glycoprotein E and I of herpes simplex virus type 1. J. Virol. **64:**2181–2186.
4. **Cai, W., B. Gu, and S. Person.** 1988. Role of glycoprotein B of herpes simplex virus type 1 in viral entry and cell fusion. J. Virol. **62:**2596–2604.
5. **Cai, W., S. Person, S. C. Warner, Z. Zhou, and N. DeLuca.** 1987. Linker-insertion nonsense and restriction-site deletion mutation of the gB glycoprotein gene of herpes simplex virus type 1. J. Virol. **61:**714–721.
6. **Chatterjee, S., J. Koga, and R. J. Whitley.** 1989. A role of herpes simplex virus type 1 glycoprotein E in induction of cell fusion. J. Gen. Virol. **70:**2157–2162.
7. **Chen, C., and H. Okayama.** 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. **7:**2745–2752.
8. **Davis-Poynter, N.** Unpublished data.
9. **Dingwell, K. S., C. R. Brunetti, R. L. Hendricks, Q. Tang, M. Tang, A. J. Rainbow, and D. C. Johnson.** 1994. Herpes simplex virus glycoproteins E and I facilitate cell-to-cell spread in vivo and across junctions of cultured cells. J. Virol. **68:**834–845.
10. **Forrester, A., H. Farrell, G. Wilkinson, J. Kaye, N. Davis-Poynter, and T. Minson.** 1992. Construction and properties of a mutant of herpes simplex virus type 1 with glycoprotein H coding sequences deleted. J. Virol. **66:**341–348.
11. **Gompels, U., and A. Minson.** 1986. The properties and sequence of glycoprotein H of herpes simplex virus type 1. Virology **153:** 230–247.
12. **Herold, B. C., D. Wu Dunn, N. Soltys, and P. G. Spear.** 1991. Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. J. Virol. **65:**1090–1098.
13. **Hu, X., R. Ray, and R. W. Compans.** 1992. Functional interactions between the fusion protein and hemagglutinin-neuraminidase of human parainfluenza viruses. J. Virol. **66:**1528–1534.
14. **Johnson, D. C., M. C. Frame, M. W. Ligas, A. M. Cross, and N. D. Stow.** 1988. Herpes simplex virus immunoglobulin G Fc receptor activity depends on a complex of two viral glycoproteins, gE and gI. J. Virol. **62:**1347–1354.
15. **Ligas, M. W., and D. C. Johnson.** 1988. A herpes simplex virus mutant in which glycoprotein D sequences are replaced by β-galactosidase sequences binds to but is unable to penetrate into cells. J. Virol. **62:**1486–1494.
16. **MacLean, C. A., S. Efstathiou, M. L. Elliott, F. E. Jamieson, and D. J. McGeoch.** 1991. Investigation of herpes simplex virus type 1 genes encoding multiply inserted membrane proteins. J. Gen. Virol. **72:**897–906.
17. **MacLean, C. A., L. M. Robertson, and F. E. Jamieson.** 1993. Characterization of the UL10 gene product of herpes simplex virus type 1 and investigation of its role *in vivo*. J. Gen. Virol. **74:**975–983.
18. **Minson, A. C., T. C. Hodgman, P. Digard, D. C. Hancock, S. E. Bell, and E. A. Buckmaster.** 1986. An analysis of the biological properties of monoclonal antibodies against glycoprotein D of herpes simplex virus and identification of amino acid substitutions that confer resistance to neutralisation. J. Gen. Virol. **67:**1001–1013.
19. **Neidhardt, H., C. H. Schroder, and H. C. Kaerner.** 1987. Herpes simplex virus type 1 glycoprotein E is not indispensable for viral infection. J. Virol. **61:**600–603.
20. **Peeters, B., N. De Wind, M. Hooisma, F. Wagenaar, A. Gielkens, and R. Moormann.** 1992. Pseudorabies virus envelope glycoproteins gp50 and gII are essential for virus penetration, but only gII is involved in membrane fusion. J. Virol. **66:**894–905.
21. **Sakai, Y., and H. Shibuta.** 1989. Syncytium formation by recombinant vaccinia viruses carrying bovine parainfluenza 3 virus envelope protein genes. J. Virol. **63:**3661–3668.
22. **Schranz, P., H. Neidhardt, C. H. Schroder, and H. C. Kaerner.** 1989. A viable HSV-1 mutant deleted in two non essential major glycoproteins. Virology **170:**273–276.
23. **Shieh, M.-T., and P. G. Spear.** 1993. Herpesvirus-induced cell fusion that is dependent on cell surface heparin sulfate or soluble heparin. J. Virol. **68:**1224–1228.
24. **Spear, P. G.** 1993. Membrane fusion induced by herpes simplex virus, p. 201–232. *In* J. Bentz (ed.), Viral fusion mechanisms. CRC Press, Inc., Boca Raton, Fla.
25. **Weise, K., H. C. Kaerner, J. Glorioso, and C. H. Schroder.** 1987. Replacement of glycoprotein B gene sequences in herpes simplex virus type 1 strain ANG by corresponding sequences of the strain KOS causes changes of plaque morphology and neuropathogenicity. J. Gen. Virol. **68:**1909–1919.
26. **Wilson, D. W., N. Davis-Poynter, and A. C. Minson.** 1994. Mutations in the cytoplasmic tail of herpes simplex virus glycoprotein H suppress cell fusion by a syncytial strain. J. Virol. **68:**6985–6993.
27. **Zezulak, K. M., and P. G. Spear.** 1982. Mapping of the structural gene for the herpes simplex virus type 2 counterpart of herpes simplex virus type 1 glycoprotein C and identification of a type 2 mutant which does not express this glycoprotein. J. Virol. **49:**741–747.
28. **Zsak, L., F. Zuckermann, N. Sugg, and T. Ben-Porat.** 1992. Glycoprotein gI of pseudorabies virus promotes cell fusion and virus spread via direct cell-to-cell transmission. J. Virol. **66:**2316–2325.